

Acta Bot. Croat. 49, 41—46, 1990.

CODEN: ABCRA2
YU ISSN 0365—0588UDC 581.165.7:582.632.1 = 20
Original scientific paper

IN VITRO MULTIPLE SHOOT INDUCTION AND PLANT REGENERATION IN *BETULA PENDULA*

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Received December 28, 1989

Shoot development and plant regeneration through axillary bud culture of the European birch (*Betula pendula* Roth.) were successfully achieved. The modified ACM medium supplemented with 2.2 μM BA was used as initial medium. Both adventitious and axillary shoot development was induced in 2-year-old genotype explants, while in 4-year-old genotype cultures only axillary shoots developed. The establishment of multiple shoot culture was genotype depending. It was found that ACM nutrition medium, supplemented with 2.2 μM BA, was more effective for shoot multiplication than WPM and BTM. Microcuttings were easily rooted on ACM medium supplemented with 1.0 μM IBA. The complete plants obtained were successfully transferred to soil.

Introduction

The European birch (*Betula pendula* Roth.) has been identified as potential woody bioenergy crop and a species with positive influence on soil pH. It is known that vegetative propagation of species in the genus *Betula* by cuttings is of limited success (Suzska 1979) which is the main problem for foresters in mass propagation of selected trees. The application of tissue culture methods offers new prospects for their rapid multiplication. In recent years numerous studies on *in vitro* propagation of woody plants have shown that these techniques may be a solution for rapid propagation of selected forest trees (Chalupa 1987, McCown and McCown 1987, Boulay 1987). Moreover, the microshoots ob-

tained by these methods could be the material source for studying the morphogenetic capacity of different organs and tissues, appearance of somaclonal variants (Besendorfer et al. 1989) and resistance to pests or diseases. *In vitro* regenerated plants of some species in the family *Betulaceae* were used for studying the micorrhizal formation *in vitro* (Perinet and Lalonde 1983, Grellier et al. 1984, Tremblay and Lalonde 1984).

The investigations on tissue culture in the genus *Betula* has been reported by Chalupa (1981, 1983), McCown and Amos (1979) and Welander (1988) but the genetic effect on the morphogenetic capacity was not discussed. It is well known that the genotype as well as the physiological condition of the explants is a very important factor determining the possibility of vegetative propagation *in vitro* as well as *in vivo*.

In the present investigation we have tried to work out not only an *in vitro* method, but also to establish how the genotype and the age of donor plants affected shoot multiplication and plant regeneration of *Betula pendula* Roth.

Material and Methods

Plant material

Axillary buds of 2-year-old (genotypes JV₁, JV₂, JV₃) and 4-year-old (genotypes M₁, M₃, M₄) plants of *Betula pendula* Roth. were used as primary explants. The buds were excised from twigs collected in February to March. Dormant buds were surface sterilized by being agitated in 3% chlorine product Izosan-G for 20 minutes and then in 6% hydrogen peroxide for 5 minutes. After sterilization the buds were washed in sterile distilled water.

Media composition

Modified Aspen Culture medium, ACM (Ahuja 1983), without adenin sulfate, with 342 μ M lysine and 2.2 μ M 6-benzylaminopurine (BA) was used as induction medium.

The following three media: mod. ACM, Woody Plant Medium, WPM (McCown and Lloyd 1981) with 200 μ M Na₂EDTA and FeSO₄·7H₂O, and Broadleaved Tree Medium, BTM (Chalupa 1983), supplemented with 2.2 μ M BA, were used for shoot multiplication.

Microcuttings were rooted on ACM medium supplemented with 1.0 μ M indole-3-butyric acid (IBA).

Sucrose (2%) and Bacto-agar (0.9%) were added to all the media. The pH was adjusted to 5.7–5.8 before autoclaving.

Culture conditions

Cultures were kept in an air-conditioned chamber at temperature of 25±1°C under the illumination of fluorescent lamps (800–1500 lx) and during a light-dark cycle of 16–8 hours.

As a potting substrate the horticultural mixture of peat/perlite was used.

Results and Discussion

Culture initiation and establishment

After removal of the scales, single explants were inoculated on the induction medium, mod. ACM supplemented with 2.2 μM BA. The development of axillary shoot was induced in about 60% of the bud cultures. Within 4 weeks the elongated shoots were 1–2 cm high with 3–4 leaves. The response of explants on the culture condition was depended on genotype (Tab. 1). The genotype differences in the response

Table 1. Shoot development in birch bud explants in correlation to the genotype and the age of donor plant.

Genotype	Age of stock plant	No. of inoculated buds	% of buds developed in shoots
JV ₁	2-year-old	8	75.0
JV ₂		8	50.0
JV ₃		8	80.0
total		24	67.0
M ₁	4-year-old	32	47.0
M ₃		26	73.0
M ₄		19	80.0
total		77	64.0

of the explants cultured *in vitro* have been reported in related species *Alnus* and in *Salix* (Read et al. 1982, Farnham et al. 1982). The age of the donor plants was not a limiting factor for shoot induction.

The basal region of inoculated buds produced a large amount of green callus on the low concentration of BA (2.2 μM). The highest callus growth was noticed in the bud culture of 2-year-old genotypes (JV₁, JV₂, JV₃). At the same time, in the basal callus of these genotypes the adventitious shoots appeared (Fig. 2). Axillary and adventitious shoots were morphologically different. The axillary shoots were vigorous with short internodes and large leaf surfaces, whereas the adventitious shoots were thinner and had small leaf shapes. The same morphological differences between two kind of shoots in European birch were described by Welandner (1988). The formation of adventitious buds in the basal callus in the culture of 4-year-old genotypes (M₁, M₂, M₃) was not noticed. Developed shoots were obtained only as the results of axillary branching of the main shoot. Abbott and Whiteley (1986) described this phenomenon in apple culture. They found that mixed cultures of axillary and adventitious shoots appeared only when buds from juvenile plants were used.

Stem segments with 2–3 axillary buds have been transferring to the same composition medium in 4–5 week intervals. During subcultivation the morphological characteristics of the cultures noticed in the primary culture were retained. Multiple shoot cultures (Fig. 3) were obtained with 100 per cent success in the tested genotypes, except JV₂. After the fourth subcultivation the number of JV₂ cultures with developed shoots decre-

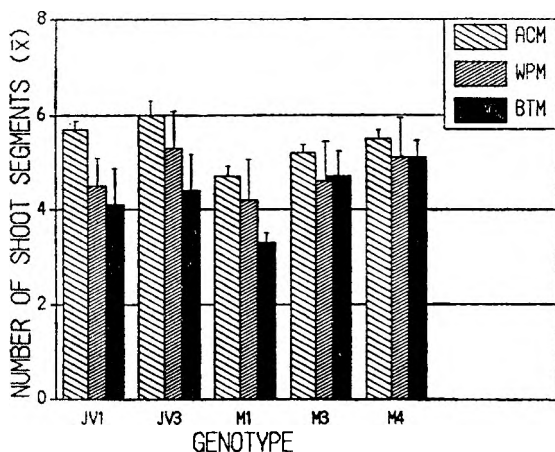


Fig. 1. The effect of genotype and composition of basal medium ACM, WPM and BTM on the multiplication rate in multiple shoot culture of *Betula pendula*. Picture shows average data collected during 6–8 months in culture.

used while the shoot development in the seventh subculture was not induced. Our results showed that maintenance of multiple shoot culture was affected by the genotype.

Shoot multiplication

During 6–8 months, the multiplication rate, presented as a number of shoot segments per inoculum, was investigated. The rate of multiplication was analysed on ACM, WPM and BTM media supplemented with 2.2 μM BA. Shoot multiplication was possible on all the three media tested, but the ACM medium seemed to be superior to WPM and particularly in relation to BTM (Fig. 1). Chalupa (1983) reported the WPM and BTM media supplemented with a similar concentration of BA, were suitable for rapid clonal propagation of birches. In our investigation the multiplication rate achieved on the mentioned media was lower. The shoots obtained on WPM medium were yellowish green. When the concentration of Na_2EDTA and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was doubled (200 μM) normal green-leaved shoots developed.

The genotypic effect on the multiplication rate was noticed in all tested media, but the differences between genotypes were not significant (Fig. 1). We also observed that the age of the stock plants used in our investigations was not a limiting factor for rapid multiplication. Results obtained could be explained by the strong effect of ecotypes as well as genotypes as suggested by Farnham et al. (1982) in their work with *Alnus rubra*.

Rooting of shoots

Microshoots excised from the shoot multiplication culture were rooted on the ACM medium supplemented with 1.0 μM IBA. Rooting of 100% of the microcuttings and complete plant regeneration were

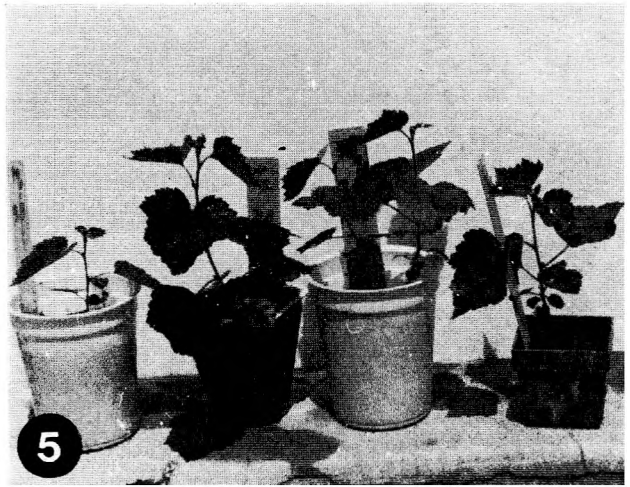
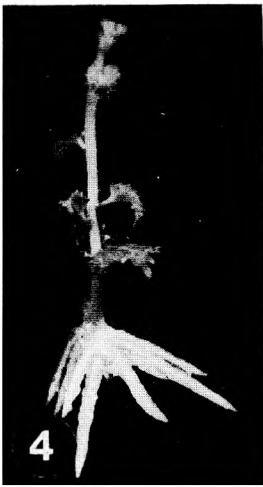
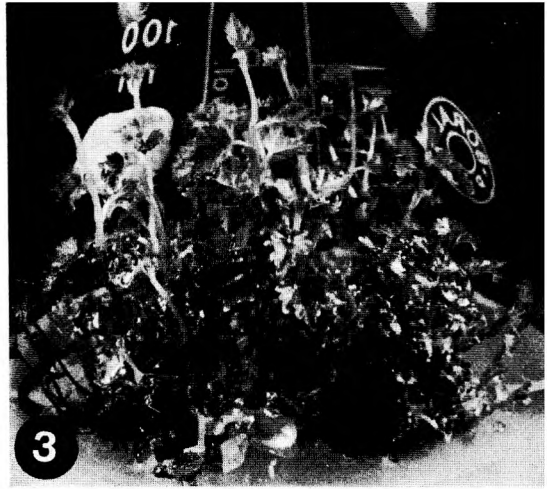


Fig. 2. The elongation of axillary shoot (white arrow) and adventitious shoot development (black arrow) in basal callus of the explant in primary bud culture, genotype JV_3 , on ACM medium supplemented with $2.2 \mu\text{M}$ BA.

Fig. 3. Multiple shoot culture of *Betula pendula*, genotype JV_3 , on modified ACM medium supplemented with $2.2 \mu\text{M}$ BA.

Fig. 4. Complete plants, genotype M_4 , regenerated *in vitro*. The rooting of microcuttings obtained on ACM medium supplemented with $1.0 \mu\text{M}$ IBA.

Fig. 5. Plantlets after 2 months of acclimatization and greenhouse cultivation.

accomplished on this medium after 10 days (Fig. 4). The successful root induction using low concentration of IBA was described by Chalupa (1981, 1983) and Perez and Postigo (1989) in cultures of the same or related species.

The regenerated plants were transferred to an artificial horticultural peat/perlite substrate and subsequently acclimatized to greenhouse conditions (Fig. 5). After 2—3 months of cultivation in a greenhouse the plantlets were successfully transferred to a field.

Our results indicate that there is a considerable potential for rapid *in vitro* propagation of *Betula pendula* when using the multiple shoot culture method. With a proper selection of the genotype and nutrition media it has been possible to produce complete plants. The high efficiency of this method suggests the possibility of its commercial utilization.

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Acknowledgments. This research was supported by the Science Research Council of SR Croatia (Research Project No. 2. 04. 01. 03. 17 and the Federal GIBIT Grant No. 1.64.1.).

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SAŽETAK

INDUKCIJA MULTIPLIH IZDANAKA I REGENERACIJA BILJAKA VRSTE *BETULA PENDULA IN VITRO*

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Razvitek multiplih izdanaka i regeneracija biljaka dobivena je kulturom aksilarnih pupova obične breze (*Betula pendula* Roth.). Za indukciju izdanaka korištena je modificirana hranidbena podloga ACM uz dodatak 2,2 μM BA. Kod dvogodišnjih genotipova za razliku od četve-rogodišnjih, inducirani su osim aksilarnih i adventivni izdanci. Mogućnost uspostavljanja kulture multiplih izdanaka ovisila je o genotipu. Utvrđeno je da je podloga ACM s 2,2 μM BA u odnosu na WPM i BTM s istom koncentracijom citokinina bila pogodnija za umnažanje izdanaka. Izdanci su uspješno zakorjenjivani na ACM hranidbenoj podlozi uz dodatak 1,0 μM IBA. Kompletne biljke uspješno su se prilagodile na vanjske uvjete uzgoja.

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